THE COMPOSITION OF CHLOROPHYLL

AMONG recent papers on chlorophyll those of Hansen, assistant to Prof. Sachs, are worthy of notice.[1]

Dr. Hansen has applied the saponification method, found so useful by Prof. Kühne[2] in his researches on the chromophanes, to the study of chlorophyll, and has been led to some very important conclusions. It may be remembered that Fremy tried to show that the green chlorophyll colouring matter consists of a blue and a yellow constituent. He mixed an ethereal chlorophyll solution with hydrochloric acid, when two layers formed—a lower blue layer and an upper yellow ethereal layer. The blue colouring matter was named by Fremy *phyllocyanin* and the yellow *phyll·xanthin*.

Hansen shows that this is not due to a splitting up of the chlorophyll green into a blue and a yellow component, but only an incomplete separation of the chlorophyll green from the chlorophyll yellow, the former becoming changed to blue by the hydrochloric acid, and he further shows that an ethereal solution of pure chlorophyll green treated with hydrochloric acid does *not* furnish any yellow constituent, the ethereal layer remaining colourless. Fremy himself, however, abandoned the view that chlorophyll consists of two colouring matters.

The views of Kraus are so well known that it is hardly necessary to recapitulate them here, but I may be permitted to recall to mind that he supposed he had decomposed chlorophyll green into a blue green and a yellow component. He mixed an ordinary alcohol chlorophyll solution with benzol, and obtained two layers, an underlying yellow alcoholic layer and an upper blue-green layer. The blue-green Kraus named *cyanophyll*, the yellow *xanthophyll*.[3] Hansen shows, however, that Kraus is wrong in supposing that a *decomposition* of the green colouring matter into a blue-green and a yellow has taken place, as it is only an *incomplete separation* of the chlorophyll green from the chlorophyll yellow. Kraus's *cyanophyll* therefore is nothing more than an ordinary chlorophyll solution out of which a part of the yellow colouring matter has been removed. Both Fremy and Kraus were correct in assuming that a yellow and a green constituent were present, but incorrect in supposing they existed *in combination;* the correct view now is that *they exist side by side.* In other words, chlorophyll is merely a *mixture* of these colouring matters.[4] [I think it necessary here to give Conrad's view, viz. that Kraus had effected a decomposition of the chlorophyll by the use of water, as Kraus used weak alcohol Conrad showed that by using strong alcohol no yellow pigment could be got into solution by means of benzol. Cf. Sachs' "Botany," 2nd English ed. p. 760.] In the preparation of pure chlorophyll Hansen used young plants of wheat at the time of their growth when the fourth leaf is formed. Then the plant contains only protoplasm, chlorophyll, and cellulose. The spectra of the solutions were observed as in the experiments of Kühne,[5] on the pigments of vertebrate eyes, and of Krukenberg[6] on those of various animals, by means of sunlight thrown into the slit by a heliostat, a large chemical spectroscope having been used.

The leaves of the plants are first boiled to remove extractives, the water poured off, and the material washed with water until the wash water is quite clear. It is then quickly dried at a low temperature, and afterwards extracted with alcohol. Hansen states that the boiling does not alter the chlorophyll, since the plant residue, after boiling, gives the same bands as the living leaf. For the alcoholic extraction 96 per cent. alcohol was used, and it was carried on in a dark room to avoid decomposition of the chlorophyll by light. A second extraction was also carried out, and the alcohol left in contact with the residue until the former assumed a dark green colour.

The united alcoholic solutions were then concentrated and *saponified.* Hansen had previously found that he could separate out, in the case of the colouring matter of blossoms, by means of saponification, the yellow colouring matters from the fats in combination with them, as Kühne had previously done in the case of the *chromophanes* and other pigments, and not only did he get the pigments fat-free, but also in a crystalline state.

[1] "Der Chlorophyllfarbstoff," von Dr. Adolph Hansen, *Arbeiten des botan. Instituts zu Würzburg*, Bd. iii. Heft 1; and *Sitzungsberichte der physikal.-medicin. Gesellschaft.* Wurzburg, 1883. Also, *Die Farbstoffe der Blüthen und Früchte. Verhandlungen der physikalisch-medicinischen Gesellschaft zu Wurzburg*, N.F., Band xviii. No. 7, 1884.

[2] Kühne, *Untersuch. a. d. physiologischen Institute der Univ. Heidelberg*, Band i. Heft 4, 1878, and Band iv. Heft 3, 1882.

[3] "Zur Kenntniss der Chlorophyllfarbstoffe," &c., Stuttgart, 1872.

[4] *Ibid.* [5] *Loc. cit.*

[6] Krukenberg, "Vergleichend physiol. Studien," 1880-82.

The saponifying was carried out as follows:—The leaf-extract (alcoholic), after concentration, was treated with caustic soda solution in not too great amount, but the amount to be added has to be determined by the quantity of chlorophyll present. As a general rule Hansen used 40–50 c.c. (of a $1NaHO$ to $5H_2O$ solution) to $2\frac{1}{2}$ c.c. chlorophyll solution obtained by concentration of 16–20 c.c. alcohol extract. When the alcoholic solution boils, the caustic soda is added drop by drop, the liquid being stirred. After the alcohol is driven off, water is poured in, and the heating continued. After the evaporation of a great part of the water, alcohol is added once more, and the saponification is ended. When the alcohol has evaporated, the soap lees is diluted with water and an excess of chloride of sodium added to separate the soap, which precipitates in a granular form. It is then shaken in a separating funnel with petroleum ether, which assumes a dark yellow colour, since it removes only the yellow constituent; this extraction is repeated as long as the petroleum ether is coloured. On evaporation of the latter, the *yellow* constituent is left.[1]

The soap is now treated with ether, which removes various impurities, and a little colouring matter, and then with ether containing alcohol, which removes the *green* constituent from the soap.

Hansen asserts, and gives his reasons for the assertion, that no change takes place in the pigments by the above treatment.

The *yellow* constituent crystallises in dark yellow needles out of the petroleum ether, and gives all the reactions of a lipochrome, both as regards spectrum and chemical characters.

The *green* constituent can be obtained out of the ether-alcohol solution after occasional filtering and evaporation of the ether, and any yellow colouring matter adhering to it can be removed with petroleum ether. For the usual reactions this pigment answers very well, but for further study it has to be purified from water, &c., which is done by further treatment with ether-alcohol solution. Finally the pigment crystallises out in spherical crystals, which show a beautifully-marked cross with crossed Nicols. Even a drop of the solution allowed to evaporate on a microscopic slide allows the crystals to be seen, thousands of small "sphærocrystals" appearing on the evaporation of the ether. Hansen shows that the idea that plants contain but a small quantity of chlorophyll is erroneous, as he has obtained out of 450 grams dried wheat leaves as much as three to four grams solid colouring matter.

Chlorophyll green is opaque in the solid state, and appears of a black-green colour, and in that state possesses no fluorescence, but in solution possesses the usual red fluorescence. Its various chemical characters are given at length in the original paper, and it is shown that some of the changes with acids described by authors are not due to their action on pure chlorophyll green, but on other unknown bodies. It is free from sulphur and from iron. The elementary analyses agree very closely, and calculated for the ash-free substance are the following:—

	I.		II.
C.	67·26 per cent.	...	67·94 per cent.
H.	10·63 ,,	...	10·36 ,,
O.	16·97 ,,	...	16·12 ,,
N.	5·12 ,,	...	5·55 ,,
	99·98		99·97

The amount of carbon is 1 per cent. too low in both cases.

Chlorophyll yellow occurs in small quantity as compared with chlorophyll green, in the proportion of about 1 to 100. Its solutions show no fluorescence, and statements to the contrary have been based upon deductions drawn from imperfect methods of separation. It possesses the reactions of Krukenberg's *lipochromes*,[2] in the solid state, namely: a blue coloration with sulphuric acid, the same with nitric acid, and a green-blue with a mixture of iodine in potassium iodide. It shows three bands in the blue half of the spectrum, but no absorption of red, and agrees in spectrum with the yellow colouring matter of etiolated leaves (*etiolin*), which is incorrectly represented by some as possessing bands in the red part of the spectrum. Chlorophyll green possesses four bands in the red half of the spectrum; they agree with the four bands of the ordinary chlorophyll solutions.

With regard to Tschirsch's "pure chlorophyll," which, it may be remembered, was described in the *Journal of the Chemical Society*, February 1884, with the remark that the writer "reserved to"

[1] Compare Kühne, *loc. cit.*, Band iv. Heft 3, 1882.

[2] Krukenberg, *loc. cit.*, "Zur Kenntniss der Verbreitung der Lipochrome im Thierreiche," Zweite Reihe, 3te Abth. 1882.

himself "the right of examining it further," Hansen observes that "it possesses all the reactions of the usual chlorophyll sauce."[1]

In a second paper[2] Hansen figures the spectra of chlorophyll green and chlorophyll yellow. His researches will, no doubt, be found useful by students of vegetable chromatology.

C. A. MacMunn

RECENT MORPHOLOGICAL SPECULATIONS[3]

II.—*The Origin of Vertebrates*

Fifteen or sixteen years ago Kowalevsky's researches on the development of Amphioxus and of Ascidians seemed to be solving the question of the origin of Vertebrates. The discovery of the larval notochord in Ascidians, and the recognition of the homology of their pharyngeal clefts with the gill-slits of Vertebrates, made it necessary to acknowledge the close relationship of the two, as had been already foreshadowed by Herbert Spencer; while the yet undisputed affinity of Ascidians to Mollusks brought Vertebrates and Invertebrates together in an unbroken line. But as new knowledge brought Ascidians closer to Vertebrates, it undermined their claims to molluscan affinities; and as the doctrine of degeneration grew up, in the hands of Dohrn and Lankester, it taught that Ascidians, and Amphioxus too for that matter, were not really *ancestors* of the higher Vertebrata, but only degenerate descendants of such ancestors, poor cousins, as it were, of the higher Vertebrates. The lines by which Vertebrates had sprung from Invertebrates, the common ancestor of Ascidians, Amphioxus, and the higher Vertebrates, had still to be sought for.

Two leading theories have been formulated, and are still under discussion. The first, identified with the names of Semper and Dohrn, maintains that the nearest allies of the Vertebrates must be looked for among the Chætopod worms, the dorsal surface and spinal cord of the former corresponding morphologically with the ventral surface of the latter, and its gangliated nerve-cord. On the second view, with which we may associate the names of Balfour and Hubrecht, we must take the ancestor of the Vertebrates to have been some segmented worm, descended from the same unsegmented types as the Chætopods, but in which the two nerve-cords, at first lateral like those of Nemertines, had coalesced dorsally instead of ventrally, to form a median nervous system.

Our discussion of the first of these theories may be made clearer if we use the words "neural" and "hæmal" instead of "dorsal" and "ventral," for the gist of the theory is that in the two groups *neural* and *hæmal* surfaces remain constant, but what is dorsal in the one is ventral in the other.

In the Chætopods, say the advocates of this theory, we have a group of regularly segmented animals, not so far specialised but that we might well conceive ancestors like them to have developed into Vertebrates; they point to the relations of the nervous, vascular, and alimentary systems, and to the development of the mesoblast, as being closely parallel in the two groups; and they try to find traces or representatives in Chætopods of such typically Vertebrate possessions as notochord, gill-clefts, and swimming-bladder.

At the very outset a difficulty arises which is perhaps the greatest the theory encounters. The mouth of Chætopods is neurally placed, and surrounded by a nerve-ring; in Vertebrates it is hæmal, and it does not pierce any part of the nervous axis. Dohrn has attempted to overcome this objection. The present mouth of Vertebrates, he says, is not identical with the Invertebrate mouth; it is a distinct and secondary structure; it arises late in development, whereas in other classes the "stomodæum" or primitive oral invagination appears very early. Moreover, in the majority of Vertebrates the mouth does not persist in the position it first appears in; it arises some way off from the anterior end of the body, and in Elasmobranchs, some Ganoids, and Myxinoids it remains there, but in all other Vertebrates it becomes terminal. If we assume, then, that the mouth in existing Vertebrates is secondary, there must have been a time when it did not exist, and when its functions were performed by another or primary mouth. It has been suggested that in the *hypophysis cerebri* or "pituitary body" we have, possibly, a remnant of this primary mouth. The hypophysis cerebri appears first as an ectodermic involution, usually arising from the stomodæum; but in the lamprey, Götte, Scott, and Dohrn have shown that it arises from the ectoderm which lies anterior to the mouth. It is here, in fact, a little pit of ectoderm, placed between those other two ectodermic pits, which are to become the nose and the mouth.

If this involution ever pierced the brain and opened upon the neural surface, the fore-brain would then be evidently homologous with the supra-œsophageal ganglion of Invertebrates, or ganglion of the præ-oral lobe. A great deal may be said for thus regarding the fore-brain as distinct from the remaining nervous system; it resembles the supra-œsophageal ganglion of the Invertebrata in its close connection with the optic and olfactory organs, and in supplying only organs of sense. There is evidence to show that the third nerve belongs to the cranio-spinal series of segmental nerves, and that the olfactory and optic nerves have a different nature. If this be so, the mid-brain, giving origin to the third nerve, would appear not to have part in the ganglion of the præ-oral lobe. The termination of the notochord directly behind the fore-brain is an additional argument in favour of the morphological distinctness of the latter structure.

Thus if we follow back the genealogical record of the Vertebrates, we find that at one period their ancestors had a mouth upon the neural surface; later, two openings into the alimentary canal appear, one on the neural and one on the hæmal surface; still later the latter gains the ascendancy, and alone remains to the present time. This secondary mouth must have arisen from some pre-existing structure; it could not have originated as a simple depression of the outer skin which deepened and ultimately fused with the alimentary canal; and the only pre-existing organs which could furnish such a passage from the exterior into the alimentary tract are the gill-slits. We must conceive this Vertebrate ancestor as an animal with an intestine which opened anteriorly by a median mouth on its neural surface, and laterally by a series of segmentally situated gill-slits. The mouth took in water, which flowed out over the gill-arches just as it does still in the lower Vertebrates. If from any reason, such as the animal lying like the modern Annelids on its neural surface, it obtained a purer supply of water by taking it in through some of the gill-slits, it is conceivable that a pair of these slits assumed that office, and that by the exercise of this power the gill-slits became gradually larger, and ultimately fused in the middle line. The suctorial power thus acquired to take in water for the purposes of respiration was also of use in obtaining food, and thus a median hæmal suctorial mouth arose, such as the Myxinoids now possess. There is much evidence to show that the ancestral Vertebrate possessed a suctorial mouth which subsequently became modified for biting, and was carried forward to the front of the head. Embryology supplies the following arguments in favour of regarding the mouth as formed from the coalescence of a pair of gill-slits. It lies close against the gill-slits, it is separated from them by a gill-arch, it arises about the same time in the embryo, it opens into the alimentary canal; finally, in some Teleosteans, *Belone*, *Hippocampus*, and *Gobius*, the mouth first appears as two lateral openings, which afterwards fuse in the middle line.

Admitting that the mouth is formed of two gill-slits, we have to see from what structures in an Annelid such gill-slits could be derived. In many Chætopods no part of the body is set apart to perform the function of respiration. Where there are no gills the blood is commonly aërated in the walls of the alimentary canal, water being taken in at either end, and when charged with the waste products of respiration, it is expelled through the same opening. In some cases, as in *Hesione*, the surface with which the water comes in contact is increased by a pair of lateral sacs or diverticula. It is obvious that with such a respiratory apparatus it would be advantageous if there was an exit for the respired water distinct from its entrance, so that the blood should always be in contact with pure water. Such an exit would be formed by fusion of the respiratory diverticula with the body-wall and subsequent rupture of the latter at the points of fusion. And the apertures in the tentacles of Actiniæ and the perforated liver-diverticula of Eolis are adduced as analogous instances of such perforation.

Another suggestion which has been made to account for the origin of gill-slits is that the inner ends of some of the segmental organs gained an entrance into the alimentary tract, and, changing their function, gave rise to gill-slits.

By these steps a Vertebrate has been reduced to an Annelid structure, but certain questions which have arisen in the development of this theory remain to be answered. One is whether the

[1] Tschirsch only obtained his chlorophyll in the form of "blackish-green drops."
[2] *Loc. cit.*
[3] Continued from p. 69.